United States
Environmental Protection
Agency

Andrew W Breidenbach
Environmental Research Center
Cincinnati OH 45268

Research and Development EPA-600/D-81-113 April 1981

# EPA ENVIRONMENTAL RESEARCH BRIEF

## Electron Microscopy in Environmental Research

Richard L. Boone[1], Patrick J Clark[1,2], James R Millette[1], and Robert S. Safferman[3]

### Background

Research conducted at the U.S. Environmental Protection Agency's Cincinnati laboratories is aided by a shared electron microscope (EM) facility. This facility includes two transmission electron microscopes (TEM), a scanning electron microsope (SEM), a connecting darkroom, and a specimen preparation laboratory containing light microscopes, vacuum evaporators, and two ultramicrotomes.

Since inception, the new facility has been regulated by a committee of principal scientists representing the major program users. This shared operation, now in its eighth year, has been a complete success. In addition to enacting operating policies insuring equitable use of the facility, the committee's responsibilities also include periodic evaluation of the facility's performance; EM operator certification; acquisition of new or additional accessory equipment; and supervision of facility maintenance. The uniqueness is also reflected in the fact that each program desiring use is responsible for providing its own personnel with expertise in their specific area of interest.

The multi-faceted needs were first met with the acquisition of a JEOL* 100B TEM, which was later upgraded by the addition of a high-resolution scanning attachment. This made specimen examination possible in scanning, scanning transmission, and conventional transmission modes. To add microanalytical capability to the 100B, an energy dispersive x-ray spectrometer (EDS) was obtained, allowing elemental analysis directly from observed specimens. The recent development of even more sophisticated microanalytical instrumentation resulted in replacing the obsolete console of the ORTEC Delphi with that of the ORTEC EEDS II multichannel analyzer and data processor.

The increasing work load at the EM facility led to the acquisition of a second transmission electron microscope (the JEOL 100CX) in 1977 (Figure 1). Like the 100B TEM, the 100CX is equipped with a side-entry goniometer and an ORTEC EDS detector The EEDS II

[1]R L Boone, P J Clark, and J R Millette are with the Health Effects Research Laboratory, Cincinnati, OH 45268
[2]P J Clark was formerly with the Municipal Environmental Research Laboratory, Cincinnati, OH 45268
[3]R S Safferman is with the Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268

*The use of a specific manufactuer's name is for identification purposes only and does not constitute endorsement by the U S Environmental Protection Agency

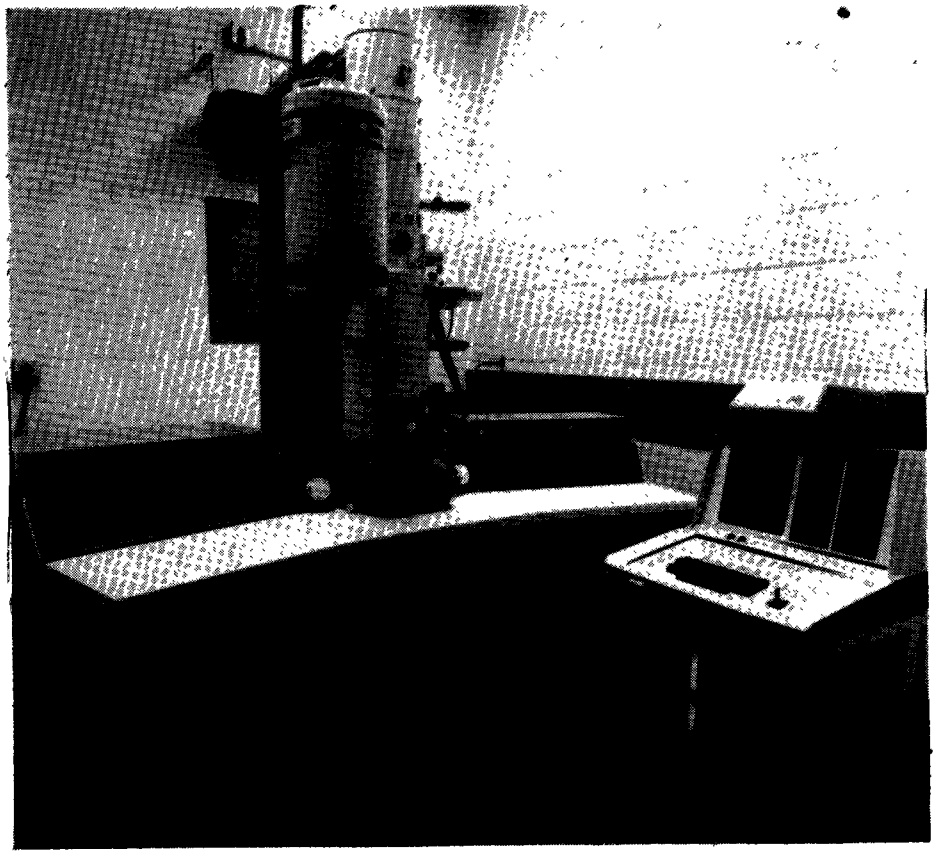

**Figure 1.** JEOL 100CX transmission electron microscope and the ORTEC EEDS II. The EM is housed in a room free from EM-interfering mechanical vibrations and magnetic fields.

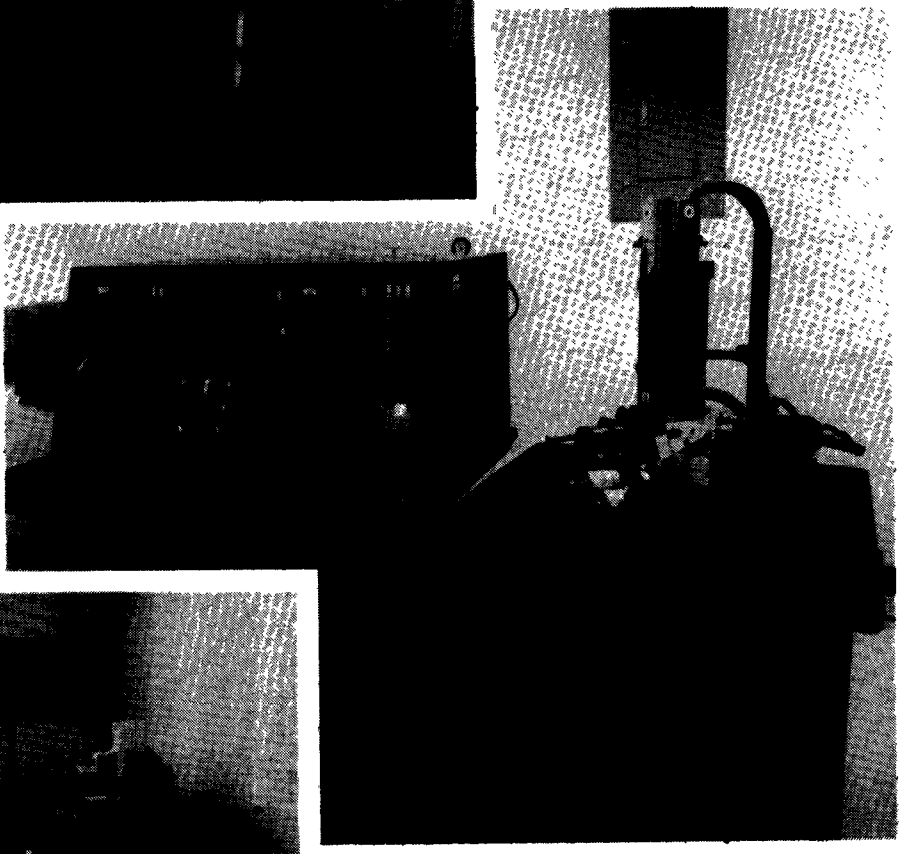

**Figure 2.** ETEC scanning electron microscope with electron column specimen chamber (right), instrument console and cathode ray tube viewing screens (left).

**Figure 3.** LKB ultramicrotome.

display console, obtained originally for the 100B, can be detached and moved to the 100CX with minor adjustments.

The facility has also added an ETEC SEM to its group of analytical tools (Figure 2). The SEM provides a three-dimensional view and an added depth of field, as well as allowing the use of relatively large samples. The SEM has recently been fitted with two viewing screens allowing simultaneous specimen observation at both low and high magnification and an EDS detector for use with the EEDS II console. The EEDS II console is now equipped to obtain data on two independent channels from separate electron microscopes. This results in faster and more efficient use of the microscopes without loss of precise analysis.

Instruments are available for conventional- and cryo-ultramicrotomy. Cryo-ultramicrotomy is used when frozen thin sectioning of tissue is needed for the microanalysis of certain elements which would be leached by usual sectioning techniques. For this purpose the LKB Ultrotome IV (Figure 3) has been fitted with an LKB CryoKit. An LKB* Ultrotome V is available for preparing conventional sections.

## Research Applications

Researchers are continually increasing their use of the EM facility for studies in the areas of methods development, air and water toxicology, and water supply. The TEM, SEM, and EDS systems have been very useful in the developmental work on asbestos standards, and also the analyses of surface waters, drinking water, minerals, volcanic ash, and microparticulates from various sources. High resolution microscopy has had a large impact on the detection of noncultivable viral agents associated with outbreaks of gastroenteritis. It is also important in inhalation toxicology studies to detect changes in tissue morphology after animal exposure to smoke and dust particulates. Moreover, research concerning ultrastructural tissue changes related to the ingestion of trace metals has been heavily dependent on EM analysis.

## Asbestos

Inhalation of asbestos fibers is a known cause of lung cancer. Ingestion of asbestos is suspect as a cause of gastrointestinal cancer among workers occupationally exposed to asbestos, and possibly may be a hazard to those who have ingested asbestos in drinking water. The TEM-EDS system is used as a complete analytical tool in the identification, characterization, and determination of the concentration of asbestos fibers in drinking water supplies throughout the United States. TEM is required because asbestos fibers which are as small as 300Å, cannot be resolved using the conventional light microscope. The exact mineral variety, elemental composition, and crystal structure are determined using TEM by direct morphology, selected area electron diffraction, and EDS (Figures 4, 5, and 6).

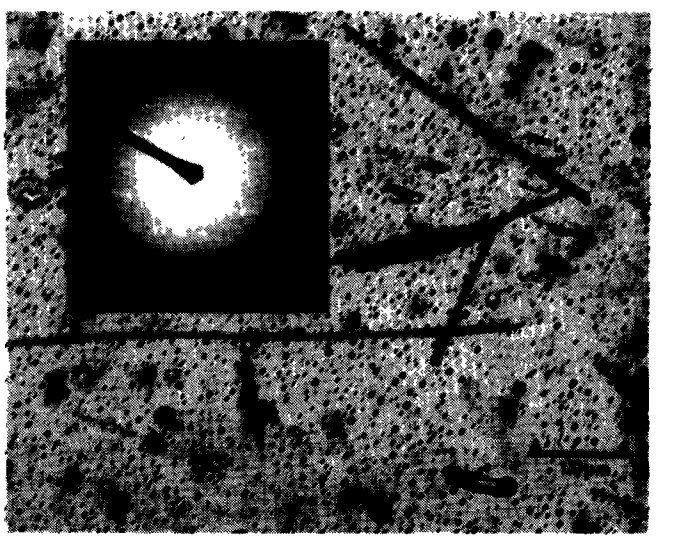

**Figure 4. Electron micrograph of chrysotile asbestos fibers in prepared standard showing distinct morphology and selected area electron diffraction pattern (upper left).**

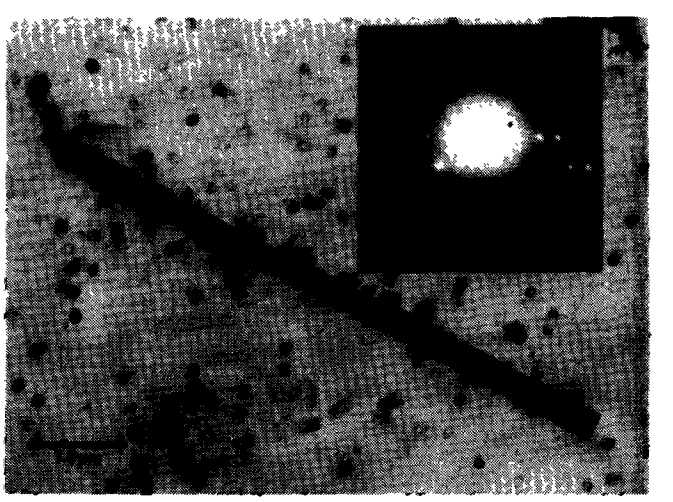

**Figure 5. Electron micrograph of amphibole asbestos fiber in water from distribution system with selected area electron diffraction pattern (upper right).**

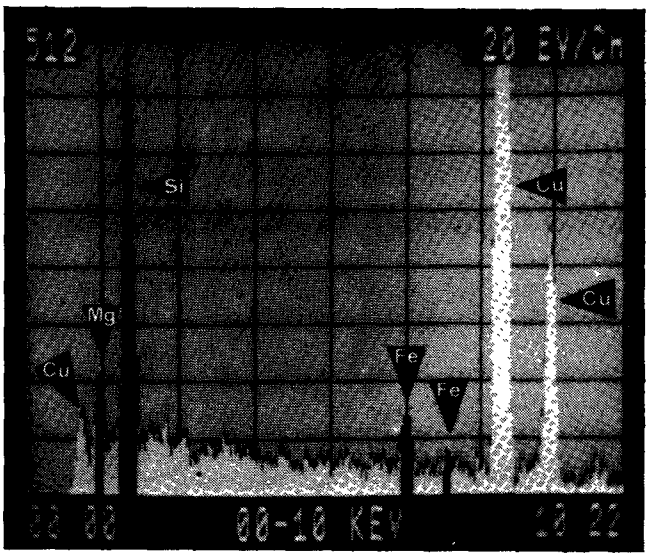


Figure 6. Characteristic spectra of chrysotile asbestos displayed on the ORTEC EEDS II console.

The SEM has been extremely useful in the examination for surface deterioration of asbestos-cement (A/C) products (water distribution pipes and roofing tiles). Continued surface deterioration commonly results in the release of asbestos fibers into drinking water supplies. Health and economic factors resulting from the deterioration of A/C materials have stimulated research on corrosion-preventive compounds, such as zinc orthophosphate. The SEM-EDS can be used to determine the coating effectiveness of these corrosion inhibitors (Figure 7a, b, and c).

Size characterization of asbestos fibers according to length and width involves EM analysis. It is believed that the size of the fiber, especially the length, may have an effect on its ability to cause cancer. It is therefore important to characterize the asbestos fibers in prepared standards used for cancer research. Once it is confirmed that a standard contains fibers of a certain size range, the effects of fiber size in the biological testing can be better ascertained. Similar characterization has also been applied to fibrous or fibrous-like clay particulates.

Monitoring drinking water for asbestos utilizes TEM analysis. Therefore, quality assurance pertaining to sample preparation and analytical technique is needed. To fulfill such a need, asbestos standards similar to known indigenous fiber concentrations and size distributions are being prepared to be sent to laboratories analyzing water samples for asbestos.

## Cardiovascular Tissue Changes

EM studies are used in the evaluation of environmental factors related to ultrastructural cardiovascular tissue changes. The ultra-microtome, modified with a CryoKit, can obtain ultra-thin frozen tissue sections of the ventricular wall of the heart, which are then analyzed by TEM-EDS. Observations are made for any soluble toxic chemicals that may have accumulated in the tissue, with special attention given to binding in the mitochondria. Morphometric analyses of electron micrographs are also being used to observe changes in the nuclear pole areas for early detection of cardiovascular disease (Figure 8).

## Virus Detection

Electron microscopy plays a crucial role in the detection of noncultivable viral agents, many of

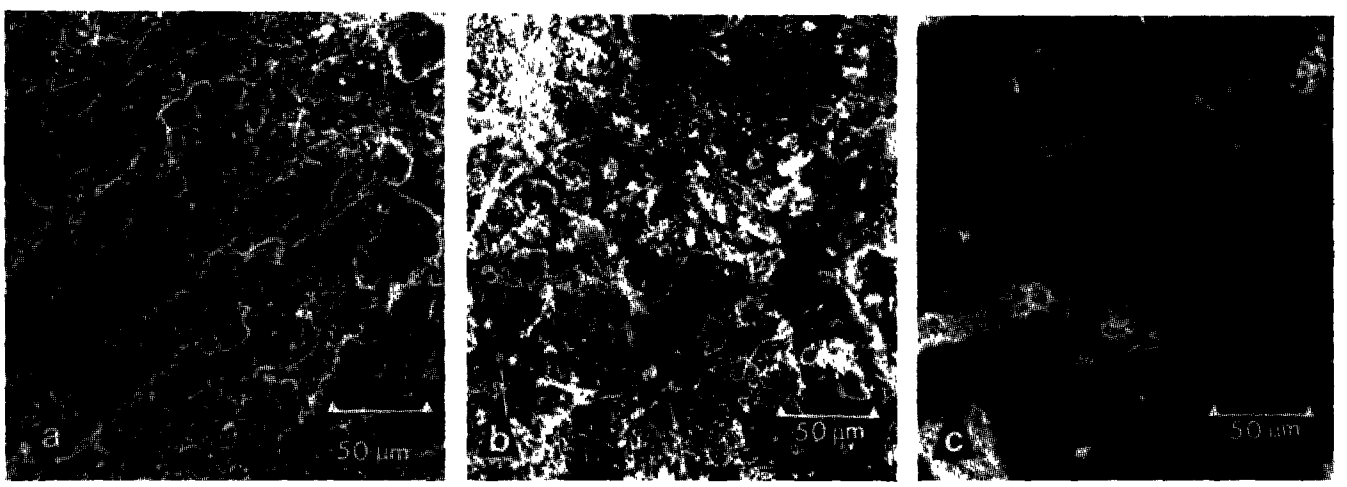


Figure 7. (a) SEM photograph of surface of unused A/C distribution pipe, (b) SEM photograph of surface of corroded A/C pipe, (c) SEM photograph of surface of A/C pipe coated with zinc orthophosphate.

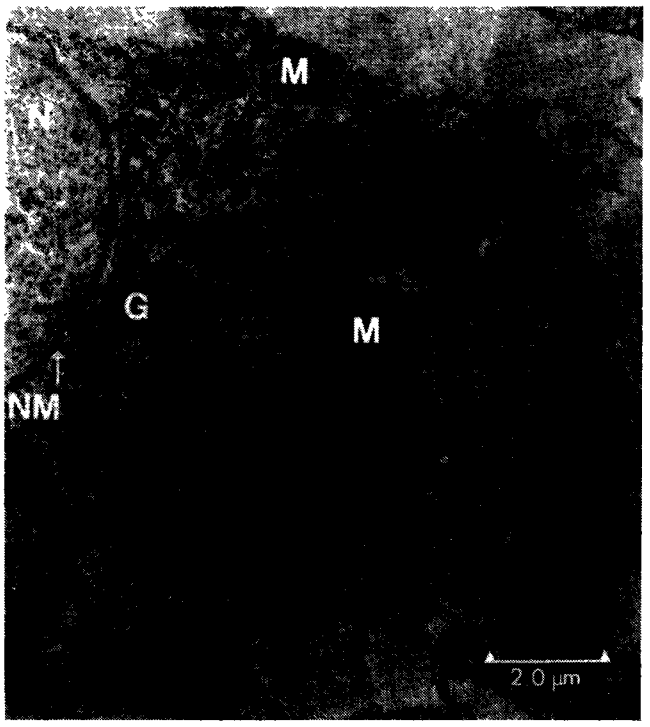


Figure 8. Electron micrograph of nuclear pole region found in rat ventricular wall. (N) nucleus; (M) mitochondria; (G) Golgi apparatus; (NM) nuclear membrane (A. Tonti).

which have been implicated in outbreaks of gastroenteritis. EM studies at this facility have detected a number of these virus particles in fecal material obtained from afflicted humans or animals (Figures 9 and 10).

## Algal Identification

Much of the structural detail of microalgae is so small it can be resolved only by TEM or SEM analyses. Both procedures have been used to examine algae from surface waters and to prepare identification manuals for field biologists to determine the effects of pollutants on the species composition of algal communities in receiving waters (Figures 11 and 12).

## Intra-Cellular Deposition of Particulates

When animals are exposed to diesel exhaust fumes, the emitted product in whole particulate form affects certain target organs of the body. The lung is a primary receptor for absorption of this particulate, which is phagocytized by selective cells. Electron microscopy magnifies the ingested particulate and the surrounding area of deposition (Figure 13). In contrast to that seen with the light microscope, this enables the laboratory to obtain a more detailed perspective of intra-cellular relationships.

## Summary

There is no doubt that the TEM, SEM, and EDS systems are powerful analytical tools for structural determination and chemical identification. These systems support important objectives of the research groups at ERC-Cincinnati through their various engineering, biological and mineralogical applications.

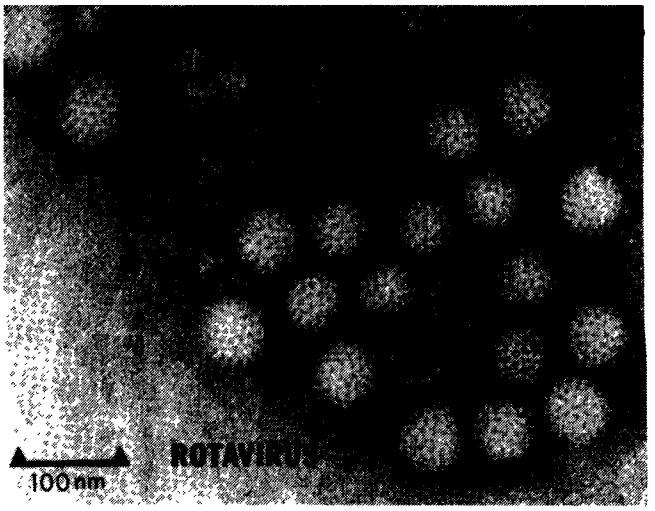


Figure 9. Rotavirus: "complete" or "smooth" double-shelled virions can be seen in the upper portion of the electron micrograph, while a group of "rough" single-shelled virions appear below. Two stain-penetrated virions of each type can be seen (F. Williams).

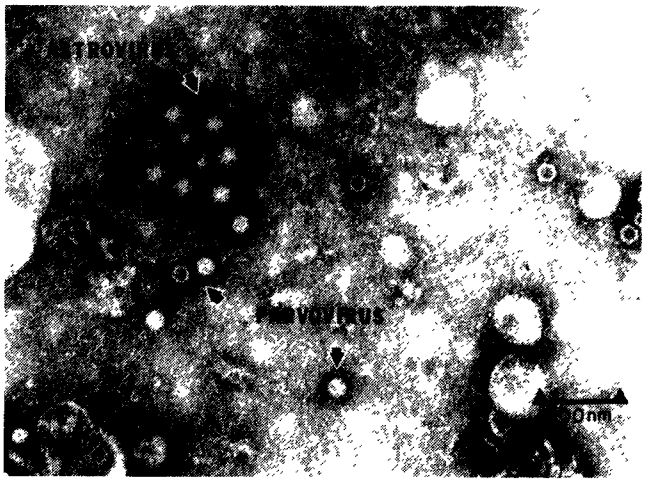

Figure 10. Electron micrograph of astrovirus particles, some possessing a distinctive star-shaped surface morphology and smaller parvovirus particles showing no such distinctive morphology (F. Williams).

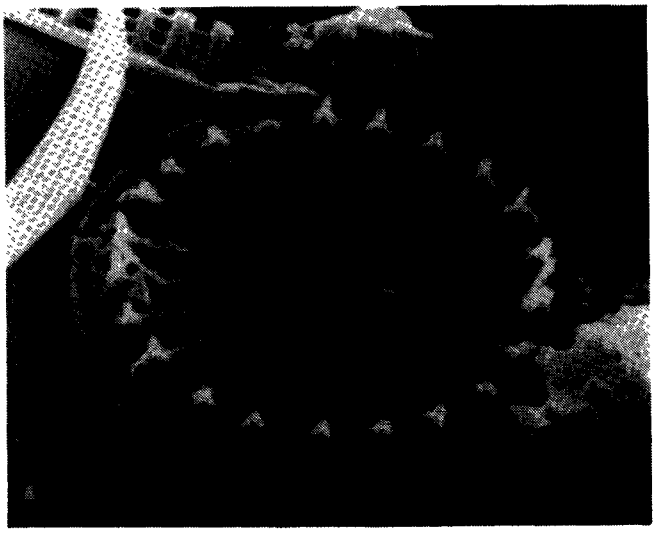

Figure 11. Scanning electron micrograph of the diatom, *Stephanodiscus hantzschii*, showing the microstructure of the cell wall (B. McFarland).

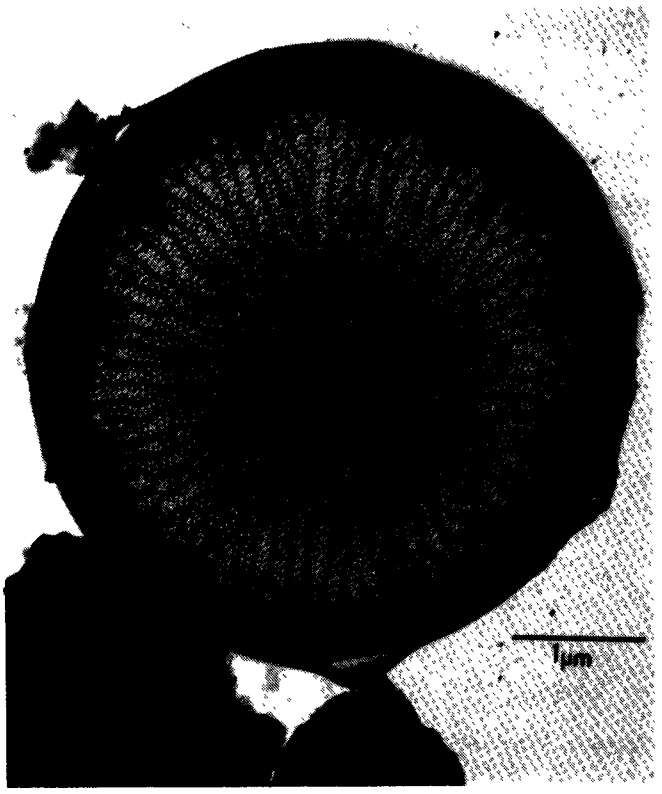


Figure 12. Transmission electron micrograph of the small diatom, *Thalassiosira pseudonana*, common in eutrophic waters (C. Weber).

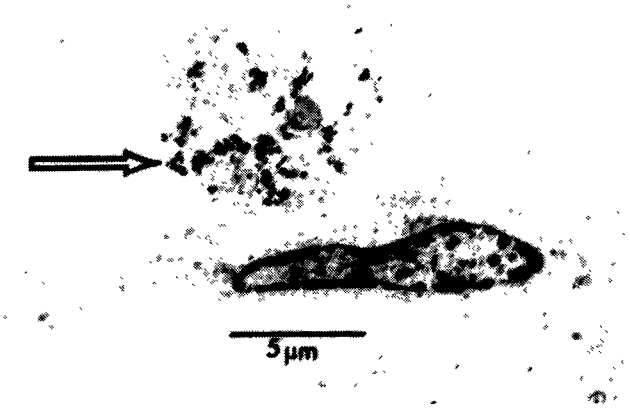


Figure 13. Transmission electron micrograph of rat lung tissue. Arrow points to a macrophage that has phagocytized deposited diesel fuel exhaust particulates inhaled into the lungs (H. Ball).



☆ U.S. GOVERNMENT PRINTING OFFICE: 1981 - 757-012/7098